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DOI: https://doi.org/10.1515/bglass-2016-0004

Research Article **Open Access**

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DOI 10.1515/bglass-2016-0004
Received Dec 09, 2015; revised Mar 02, 2016; accepted Mar 12, 2016

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Keywords: bioactive glass; radiopacity; root canal

1 Introduction

After their initial target application as bone substitutes [1], alkaline bioactive glasses of the SiO_2-Na_2O-CaO-P_2O_5 system gained the attention of dental researchers in the late 1990s [2, 3]. Alkaline biomaterials such as calcium hydroxide have a long and successful history in clinical dentistry [4]. In assimilation to the clinical usage of calcium hydroxide, the first target applications of alkaline bioactive glass powders were in the form of aqueous suspensions [2, 5]. It was shown that such suspensions act as antibacterial agents in artificially infected human root canals [6, 7]. However, in contrast to calcium hydroxide, bioactive glasses not only have antibacterial but also bioactive/remineralizing effects [8].

To combine desired bioactive glass effects with the known benefits of existing dental materials, researchers have recently started to incorporate alkaline bioactive glass particles into the matrix of dental materials such as composite resins [9], polycaprolactone [10] or guttapercha [11]. Originally, alkaline bioactive glass powders were produced from a melt. More recent attempts included the production of these materials via a one-step procedure, the so-called flame-spray process [12]. This approach produces submicron-sized particles, which have an accelerated pH induction and better initial antimicrobial effects compared to melt-derived counterparts [13]. The flame-spray process also allows incorporating extra elements into the glasses. Because the placement of prosthetic materials in teeth is monitored radiographically, flame-sprayed bioactive glass of the 45S5 type was doped with bismuth oxide [14]. It was assumed by the authors that this glass could have the advantage to be easily incorporated into the matrix of existing dental materials, without hampering their radiographic appearance. However, this assumption has not been tested thus far.

One most promising field for the application of composite dental materials containing alkaline bioactive glass

powders is endodontology. Infected root canal systems need to be treated chemo-mechanically, and then filled with a foreign material to prevent re-contamination. Incorporating bioactive glass into gutta-percha, the core material used to fill root canals [15], could afford the advantage that root canals can then be filled with one single material [10, 16]. A sealer material to connect the core filling to the root canal wall could thus become unnecessary. However, application of such a modified gutta-percha material would require the material to be heated and then applied in a thermoplastic stage into the root canal system. A so-called "cold" application of a root filling material is currently preferred by general practitioners [17], yet would still require a sealer. Among the root canal sealers, epoxy resin-based materials have stood the test of time and are among the most commonly used materials [17]. Epoxy resins work well in the inherently wet and spatially complex environment of chemo-mechanically cleaned and shaped root canal systems: unlike thermoplasticized gutta-percha they do not shrink upon setting [18, 19]. In contrast to silicone sealers, which are also popular, epoxy resin sealers bind to the collagen in the root canal wall [20]. It would thus be of clinical interest to combine the proven advantages of an epoxy resin sealer with some desired properties of bioactive glasses, particularly bioactivity and an antibacterial effect.

It was the goal of the current study to check whether alkaline 45S5 particulate bioglass can successfully be introduced into a commercially available epoxy resin sealer without hampering the microhardness of the latter after polymerization of its monomers. Furthermore, it was assessed whether nano-sized radiopaque bioactive glass particles can offer potential advantages in this context, especially better radiopacity, possibly combined with faster pH induction, or improved crystallite deposition of the resulting composite material.

2 Materials and Methods

2.1 Material preparation

Two types of bioactive glass (BG) particles were incorporated in an endodontic sealer (AH Plus, Dentsply DeTrey, Konstanz, Germany) used for root canal treatment. Micron-sized bioactive glass (MBG) was of the original composition 45S5 and had a particle size of less than 5 µm. It was purchased from a commercial source and, thus, could not be modified (Schott, Landshut, Germany). Nano-sized, radio-opaque bioactive glass (Bi-NBG) was produced by flame spray synthesis as described by Brunner *et al.* [12] with a particle size of 30–50 nm. In brief, Bi-NBG including 20 wt% bismuth oxide and 80 wt% classical 45S5 bioactive glass was prepared from the corresponding precursors [14]. AH Plus was mixed with 0, 10 or 20 wt% of each BG particles using a dual asymmetric centrifuge (SpeedMixer™ DAC 150 FVZ, Hauschild Engineering, Hamm, Germany) at 3500 rpm for 2 × 30 s. The obtained paste A and paste B of the sealer with the same amount of BG particles were mixed with a weight ratio of 1:1 using a spatula on a glass plate under clinical conditions. The mixed materials were filled into round metal molds of 8 mm diameter and 0.3 mm thickness for radiopacity assessment. For all other experiments, Teflon molds of 6 mm diameter and 1.5 mm height were used. All specimens were placed in a uniaxial press at 37°C for 24 h between polymer foils with a force of 30 kN, resulting in pressed tablets, which were used subsequently.

2.2 Radiopacity

Specimens with a thickness of 0.33–0.37 mm were placed on a radiographic sensor (Digora, Soredex, Finland) together with an aluminum step-wedge with a thickness from 1 mm to 10 mm (1 mm increments). A Trophy Irix (Trophy, Paris, France) X-ray unit operating at 65 kV, 8 mA and 0.1 s with a focus-film distance of 25 cm was used. Digital radiographic images were done in triplicates (separate image recording with aluminum step-wedge) and imported with a Digora system (Soredex), using Digora software version 2.8 for Windows. Optimas imaging analysis software (Meyer Instruments, Houston, TX, USA) was used to determine the mean gray value of the samples and to convert the mean gray values to millimeters of aluminum equivalents.

2.3 Immersion in phosphate buffered saline and simulated body fluid

To determine the *in vitro* reactions of MBG and Bi-NBG incorporated in AH Plus, the specimens were immersed in phosphate buffered saline (PBS) and simulated body fluid (SBF). SBF, which has similar ion concentrations as human blood plasma, was produced as described by Kokubo *et al.* [21] with a pH adjusted to 7.4. PBS was obtained from Gibco® (Thermo Fisher Scientific, Paisley, UK). The pressed tablets (n = 3) were placed in microcentrifugation tubes containing 1 ml of PBS or SBF for periods of 1, 7, 14 and 28 days at 37°C under static conditions

with media exchange after 7 days, respectively. After the corresponding time period, the specimens were removed, dipped in deionized water, carefully shaken and dried for one week in a desiccator (under vacuum) at room temperature.

2.4 pH measurement

Measurement of pH induction (n = 3) was performed using a calibrated pH electrode (Seven Easy, Mettler Toledo, Greifensee, Switzerland). The pH was monitored directly after sample immersion in either 1 ml PBS or SBF and after 0.5, 1, 2 h, and then 1, 2, 5 and 7 days. Every 7th day, the media were changed and the measurements repeated during a total period of 28 days. Pure PBS and SBF served as references.

2.5 Surface analysis and *in vitro* reactivity

Bioactivity can be evaluated *in vitro* by examination of surface reactions occurring in physiological fluids. Dried specimens were placed on aluminum holders (d = 12 mm) with the aid of carbon tape and sputtered for 10 s to obtain a 2–3 nm platinum layer (Cool Sputter Coater Leica EM SCD005). Before sputtering, silver paste (SPI Supplies, West Chester, PA, USA) was painted around the specimens to obtain a better conductivity. To compare the surface morphology of the as-prepared specimens to incubated counterparts (37°C in either PBS or SBF for up to 28 d), a scanning electron microscope (SEM) Nova NanoSEM 450 (FEI, Eindhoven, The Netherlands) was operated at 5 kV.

2.6 Determination of microhardness

Knoop hardness of as prepared specimens (preparation see above) after curing was measured using a digital microhardness tester (model no. 1600–6106, Buehler, Lake Bluff, IL, USA). For each specimen in each group (n = 3) three positions were selected randomly around the center of the cured specimen, where indentations were performed under a static load of 100 g for 15 s. The pits in the specimens were quantified at an optical magnification of 10x and converted into Knoop Hardness Numbers (KHN).

2.7 Data presentation analysis

Numerical data were statistically compared between groups using one-way analysis of variance (ANOVA) followed by Tukey's HSD test (JMP, version 10). The alpha-type error was set at 5% ($P < 0.05$).

3 Results

AH Plus showed a radiopacity of 13.5 mm Al (extrapolated to 1 mm material for comparison). Adding bioactive glasses to the sealer affected this parameter (supporting information). The corresponding values were significantly lower ($P < 0.05$) with 10 and 20 wt% Bi-NBG at 10.1 ± 0.1 mm and 9.8 ± 0.5 mm Al, respectively. Compared to AH Plus materials doped with the Bi-NBG, 10 and 20 wt% loading with MBG resulted in a significant ($P < 0.05$) further decrease in radiopacity. The mean values were 8.8 ± 0.1 and 7.5 ± 0.1 mm Al, for the material containing 10 and 20 wt% MBG, respectively.

Immersing the specimens in PBS resulted in a sharp pH increase for samples loaded with BG (Fig. 1). Pure AH Plus as a reference did not alter the pH (7.4) of PBS or SBF. In general, specimens containing 20 wt% BG showed a higher pH increase compared to 10 wt% BG at respective time intervals of immersion. Additionally, the pH saturation level decreased from the first week to the fourth week of immersion. Specimens in SBF showed similar behavior. However, the pH increase and saturation at the end of each week were considerably lower. AH Plus filled with Bi-NBG revealed a faster pH increase while MBG as a filler resulted in a higher final pH value for PBS as immersion liquid (Fig. 1).

The pH of Bi-NBG composites after 1 day in SBF decreased after every weekly change of the immersion medium until it reached an almost constant pH level (Fig. 2). Whilst the pH induction (bars after 1 hour in Fig. 2) slightly declined for Bi-NBG with prolonged immersion time, it did not change in the case of MBG, suggesting a higher capacity of the MBG. Compared to SBF, samples with 20 wt% BG in PBS revealed some differences. AH Plus filled with 20 wt% MBG showed the highest pH values in the second week of immersion and afterwards the pH started to decrease. For Bi-NBG the pH dropped continuously. The difference between the pH of 1 hour and 1 day in the same week changed similarly to the one as described before for Bi-NGB and MBG, respectively.

In vitro bioactivity was examined by SEM, showing the formation of apparent calcium phosphate (Ca/P) crystals on the AH Plus surface after immersion in PBS or SBF. Before immersion, the specimens showed a slightly rough surface (Fig. 3 (a1-c1) and Fig. 4 (a1-c1)), which became more even after immersion for specimens containing no

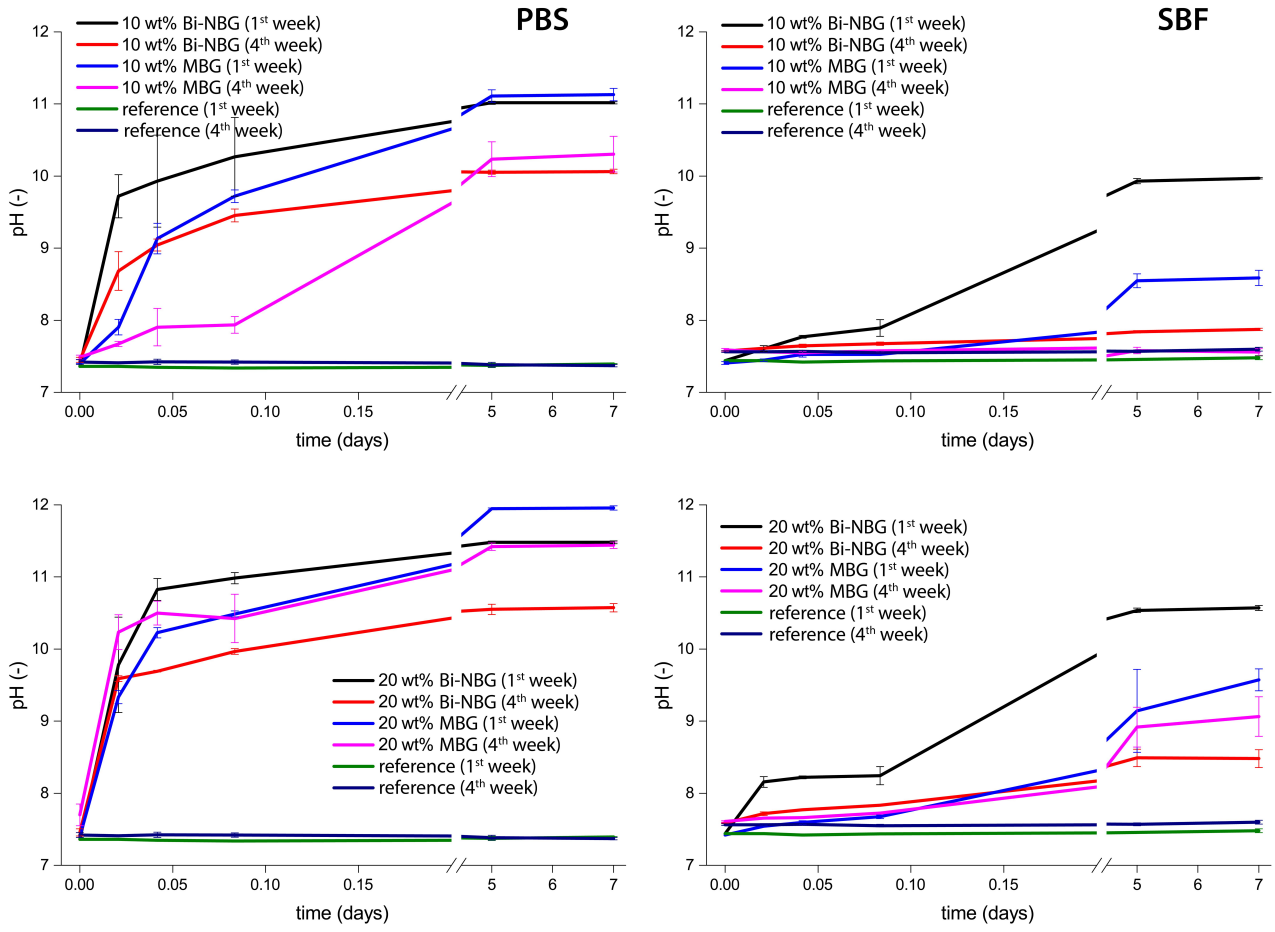

Figure 1: pH evolution of AH Plus with different bioactive glass (BG) loadings in either phosphate buffered saline (PBS, left column) or simulated body fluid (SBF, right) for the first and fourth week (0–7 days and 21–28 days). The aqueous medium was exchanged every week (weeks 2 and 3 are not shown).

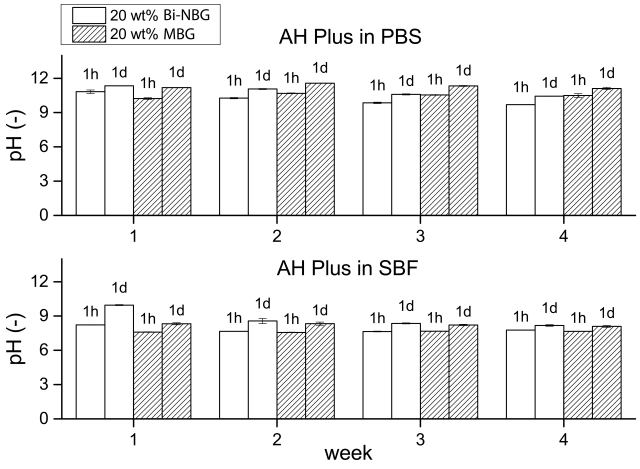

Figure 2: pH of AH Plus with 20 wt% bioactive glass (BG) loading in either phosphate buffered saline (PBS, top) or simulated body fluid (SBF, bottom). The aqueous medium was exchanged every week.

BG particles (Fig. 3 (a2-a4) and Fig. 4 (a2-a4)). In the case of BG-filled AH Plus, no BG particles could be seen on the surface of as-prepared specimens. After immersing the AH Plus/BG composites in PBS or SBF, however, they presented a tremendous surface change. Immersion for 1 day resulted in a layer of deposits on the surface, which could not cover the complete surface (Fig. 3 (b2, c2) and Fig. 4 (b2, c2)). This structure started to extend with increasing time of immersion in PBS or SBF, *i.e.* after 7 and 28 days (Fig. 3 (b3-b4, c3-c4) and Fig. 4 (b3-b4, c3-c4)).

AH Plus compounded with 20 wt% Bi-NBG (insets in Fig. 3 (b4) and Fig. 4 (b4)) revealed similar crystal structures of nanosized agglomerates, independent of the immersion medium (shown at higher magnification). MBG composites in PBS (Fig. 3 (c4)) induced a similar morphology as Bi-NBG composites but presented smaller crystallites than those observed with Bi-NBG. Fig. 4 (c4) shows that the layer of deposits on AH Plus filled with 20 wt%

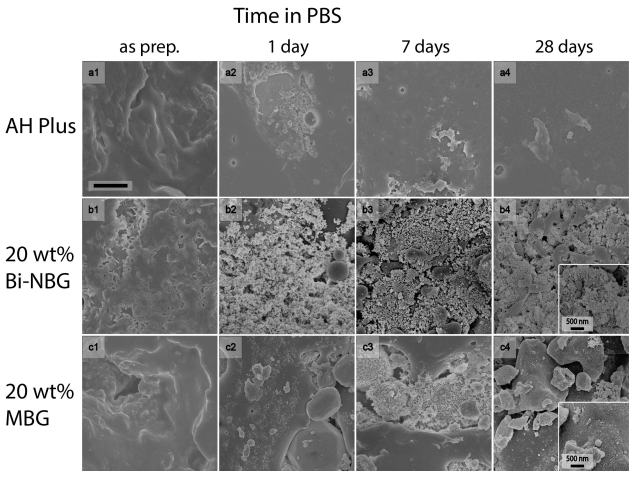

Figure 3: AH Plus with different loadings: (a) without bioactive glass (BG), (b) 20 wt% Bi-NBG and (c) 20 wt% MBG. (1) As-prepared specimens were compared to specimens immersed for (2) 1 day, (3) 7 days and (4) 28 days in phosphate buffered saline (PBS). Scale bar: 3 µm.

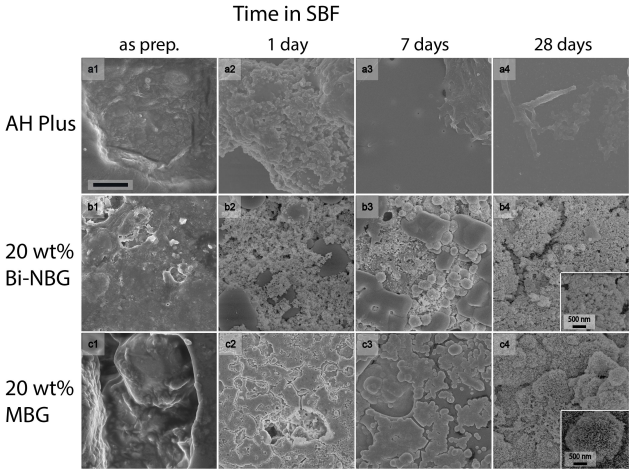

Figure 4: AH Plus with different loadings: (a) without BG, (b) 20 wt% Bi-NBG and (c) 20 wt% MBG. (1) As-prepared specimens were compared to specimens immersed for (2) 1 day, (3) 7 days and (4) 28 days in simulated body fluid (SBF). Scale bar: 3 µm.

MBG in SBF is composed of nanocrystals with a typical cauliflower structure.

KHN of BG composites increased with the amount of BG particles (Table 1). While AH Plus filled with Bi-NBG showed a somehow linear increase with particle loading, AH Plus doped with 10 wt% or 20 wt% MBG did not differ significantly ($P > 0.05$) in hardness values. As can be appreciated in Table 1, KHN of MBG composites exhibited significantly higher hardness values for a filler content of 10 wt% compared to Bi-NBG, while composites filled with 20 wt% Bi-NBG showed the significantly highest KHN.

Table 1: Knoop hardness of AH Plus with different types and amounts of bioactive glass particles.

AH Plus	0 wt%	10 wt%	20 wt%
Bi-NBG	12.4 ± 0.8^{A}	15.7 ± 0.2^{B}	22.9 ± 1.3^{D}
MBG		20.6 ± 0.3^{C}	20.8 ± 0.3^{C}

Identical superscript letters indicate that there was no statistical difference at the 0.05 level between respective data sets/groups (one-way ANOVA, Tukey's HSD).

Bi-NBG: flame sprayed bismuth-doped nanometric bioactive glass; MBG: conventional micron-sized bioactive glass.

4 Discussion

The current results suggest that alkaline bioactive glasses of the 45S5 type can be introduced into the epoxy resin matrix of a commercially available root canal sealer. Adding radiopaque, nanometric BG particles instead of conventional BG resulted in significantly less radiopacity reduction. The obtained composites induce Ca/P precipitation at their surface in aqueous environments and induce a high pH. The latter is mainly responsible for the antimicrobial effect of 45S5 bioactive glasses [22]. Consequently, the sealer materials under investigation should have a combined antimicrobial and bioactive effect while maintaining a high radiopacity. This combination is desirable in the environment of formerly infected root canal systems, which are monitored radiographically. Furthermore, the current experiments showed that nanometric bismuth-doped bioactive glass particles induce a quicker pH increase and similar Ca/P deposition compared to micrometric counterparts, and maintained a higher radiopacity.

One of the most desired properties of any dental, especially of any endodontic material is the good visibility of the material on an X-ray image. For this reason, the radiopacity of an endodontic sealer is a critical property. AH Plus presents the highest visibility for root canal sealers on X-ray images with 13.6 mm Al per mm of material according to the manufacturer's information sheet. Other studies reported lower values such as 11.2 [23] and 10.4 mm Al [24], whereas in this study 13.5 mm Al were measured. Adding particles that are less radiopaque decreases the radiopacity as observed for all tested experimental materials. However, by using BG particles that contain radiopaque properties such as Bi-NBG [14], the reduction is significantly lower than adding regular BG particles of the 45S5 type. By comparing pure AH Plus of this study and the results by Tasdemir *et al.* [24], and normalizing the values, this would still result in higher radiopacity values for 20 wt% loaded Bi-NBG composite systems compared to other commercial

root canal sealers, such as Diaket (3M ESPE, Seefeld, Germany) or Adseal (MetaBiomed, Chungbuk, Korea). Consequently, an adequate visibility of the material can still be achieved by the addition of Bi-NBG particles.

Although all AH Plus composite samples filled with BG particles increased the pH, considerable differences were noticed. The most obvious distinction was between the different media, PBS and SBF. Due to the buffered system of SBF, which contains ion concentrations close to the human blood plasma [21], the pH increased only slightly (Fig. 1, right). By the addition of Bi-NBG, compared to MBG, the pH increase was more prominent for the 1st week while it was much less during the 4th week. Similar observations were monitored for PBS as immersion medium (Fig. 1, left). However, for this liquid the increase was stronger for both types of BG particles, yet there were smaller differences between the different filler sizes. These observations are due to the changing particle size of the BG. Although the Bi-NBG has less alkaline power because of the reduced amount of Na^+ compared to MBG, it was still able to achieve a higher pH. This was caused by the reduced particle size as observed also for other BG particles [13, 25]. In contrast, MBG showed a higher capacity to induce a high pH after several "washing steps" and, thus, some "depot effect" [6]. Whether these pH differences play any role in a clinical situation remains elusive. Other groups who experimented with bioactive glasses in dental materials used different bioactive glasses, such as a sol-gel derived 63S [26, 27] or a phosphate-based bioactive glass [28]. Sodium-free materials, such as 63S, however, are merely bioactive yet poorly alkaline and, thus, have lesser antimicrobial properties compared to the 45S5-type glasses [22]. This is even the case for nanosized BG particles [27], although they are highly active due to the high specific surface area. Nevertheless, Khvostenko *et al.* have shown that the addition of low-alkaline bioactive glass fillers to composite restorations can reduce the bacterial penetration in marginal gaps, most likely because of precipitate formation therein [29].

The advantage of using nanosized BG particles as composite fillers was mainly seen in the Ca/P induction for samples immersed in PBS. The buffering effect of PBS was weaker than for SBF and a higher pH level was obtained, which also led to a Ca/P deposition. It has been reported for a similar aqueous system (TRIS buffer) that at higher pH Ca/P precipitation occurs immediately [30]. Bi-NBG raised the pH more quickly in PBS (even with a lower Na^+ content), which could also have contributed to a faster Ca/P precipitation for the AH-Plus composites containing nanosized BG particles. Although BG particles were not visible in as-prepared composite samples (Fig. 3 (b1-c1) and Fig. 4 (b1-c1)), Ca/P crystals precipitated. Similar findings have been observed for BG particles in a trans-polyisopren composite [11]. There, the hydrophilic BG particles had also no influence on the water contact angle, even with a loading of up to 30 wt%, therefore showing no exposure on the surface. However, after immersion in SBF the mentioned composites were able to form Ca/P precipitates on their surface. MBG containing composites showed typical cauliflower-like crystals after SBF immersion in comparison to smaller crystals for Bi-NBG. The addition of Bi to these BG particles could have an influence but not necessarily a distinct negative effect as shown for other radiopaque markers like Ba [31]. Formation of Ca/P crystals for Ba-containing BG was also slightly slower than for conventional BG. Similar findings were observed for Cu, Zn or Mg doping [32]. In this study, Ca/P precipitates were only shown by SEM images. Further standard techniques, such as X-Ray diffraction (XRD) or elemental mapping (EDX) would be highly desired yet not applicable in this work. This was due to the use of a commercial matrix, which included highly crystalline particles: calcium tungstate and zirconium oxide. The presence of these particles made it impossible to provide useful information from the mentioned analytical methods because zirconium oxide and calcium tungstate overlap with meaningful Ca/P peaks in the EDX or XRD. However, comparing the SEM images (Fig. 3 and Fig. 4) with recent literature [9, 33], it can be stated that the precipitations were calcium phosphates.

In accordance with previous findings on dimethacrylate-based composite materials loaded with inert inorganic filler particles [34] or bioactive glass fillers [35], in the present study, microhardness of the epoxy resin specimens increased with the per-weight filler load. The hardness increase strongly suggests a close interaction between the BG fillers and the epoxy resin matrix. This contrasts with observations made with the same fillers used in a Bis-GMA/TEGDMA matrix [9] where no increase in hardness could be observed in the set composites. The more pronounced KHN increase for the 20 wt% Bi-NBG loading could be due to the more homogenous distribution of nanoparticles at the surface compared to the micronsized BG particles [36].

Future investigations should study the effect of incorporating bioactive particles into the matrices of common endodontic sealers in more detail. The effect of such a material alteration on *e.g.* the binding properties of epoxy resin sealers to dentin and their sealability should be assessed before any pre-clinical conclusions can be drawn.

5 Conclusion

This study showed that a radiopaque nanometric bismuth-containing 45S5 bioactive glass reduces radiopacity of a commercially available epoxy resin root canal sealer less than a conventional micrometric 45S5 bioactive glass. In addition, pH induction and Ca/P precipitation was quicker with the nanometric compared to the micrometric material, whilst the micrometric glass displayed a higher alkaline capacity. Both materials apparently bound to the epoxy resin matrix, thus increasing its microhardness after polymerization of the monomers. Under the limitations of this study, Bi-NBG could be a valuable add-on for root canal sealers because of its inherent radiopacity, bioactivity and pH induction potential.

Acknowledgement: This study was supported by the authors' institutions. We would like to thank Dr. Kirill Feldman for providing access to the hot press. WJS, MZ and DM declare a financial interest in the form of a patent application (WO2011/020204) on radio-opaque bioactive glass licensed to smartodont llc., of which WJS, MZ and DM are shareholders.

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Supporting Material, Figure 1

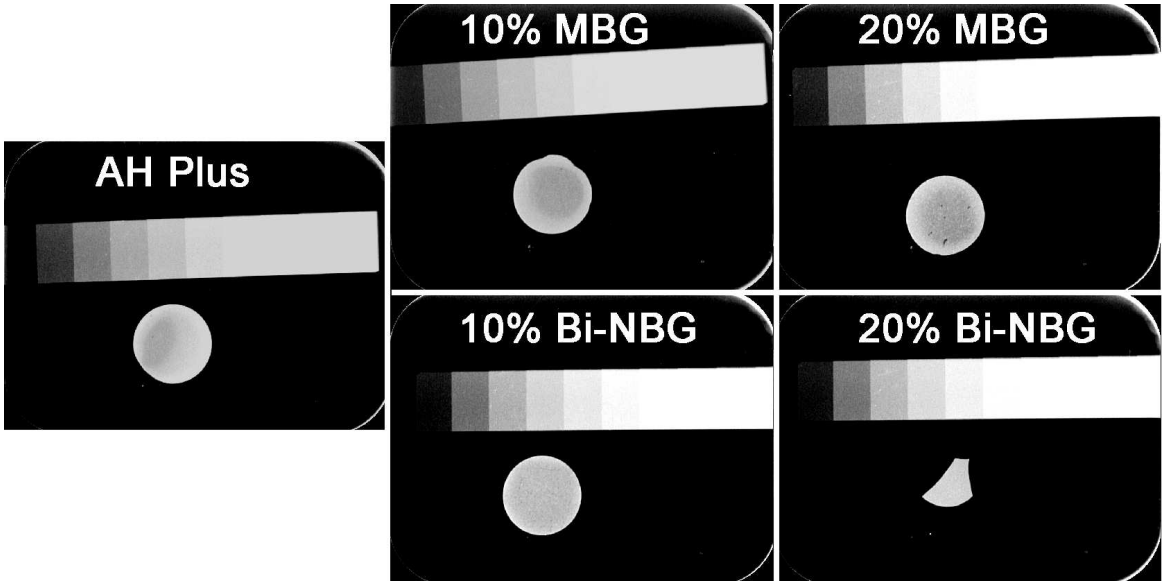

Figure 1: Radiographic images of pure AH Plus (left) and variously filled AH Plus material: micronsized bioactive glass filler (top) and nano-sized bioactive glass filler (bottom) with two different loadings. The aluminum step-wedge to evaluate the radio-opaque properties is depicted in the top part of each image. Material thickness was taken into account to express the radiopacity in mm Al per mm material.